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Monitoring poultry social dynamics using colored tags: Avian visual perception, behavioral effects, and artificial intelligence precision[☆]

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A R T I C L E I N F O

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A B S T R A C T

Artificial intelligence (**AI**) in animal behavior and welfare research is on the rise. AI can detect behaviors and localize animals in video recordings, thus it is a valuable tool for studying social dynamics. However, maintaining the identity of individuals over time, especially in homogeneous poultry flocks, remains challenging for algorithms. We propose using differentially colored "backpack" tags (black, gray, white, orange, red, purple, and green) detectable with computer vision (eg. YOLO) from top-view video recordings of pens. These tags can also accommodate sensors, such as accelerometers. In separate experiments, we aim to: (i) evaluate avian visual perception of the different colored tags; (ii) assess the potential impact of tag colors on social behavior; and (iii) test the ability of the YOLO model to accurately distinguish between different colored tags on Japanese quail in social group settings. First, the reflectance spectra of tags and feathers were measured. An avian visual model was applied to calculate the quantum catches for each spectrum. Green and purple tags showed significant chromatic contrast to the feather. Mostly tags presented greater luminance receptor stimulation than feathers. Birds wearing white, gray, purple, and green tags pecked significantly more at their own tags than those with black (control) tags. Additionally, fewer aggressive interactions were observed in groups with orange tags compared to groups with other colors, except for red. Next, heterogeneous groups of 5 birds with different color tags were videorecorded for 1 h. The precision and accuracy of YOLO to detect each color tag were assessed, yielding values of 95.9% and 97.3%, respectively, with most errors stemming from misclassifications between black and gray tags. Lastly using the YOLO output, we estimated each bird's average social distance, locomotion speed, and the percentage of time spent moving. No behavioral differences associated with tag color were detected. In conclusion, carefully selected colored backpack tags can be identified using AI models and can also hold other sensors, making them powerful tools for behavioral and welfare studies.

Introduction

Artificial intelligence (AI) is an important tool for achieving Precision Livestock Farming (Li et al., 2020, 2021a; Ojo et al., 2022) and enhancing the efficiency of experimental research programs. By pairing cameras and body-mounted sensors with AI models, animal behavior and location can be monitored remotely and automatically over extended periods. However, each technological approach has its own set of strengths and limitations. For instance, cameras paired with real-time object detection AI systems can effectively detect elements in images (e.

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g. birds), evaluate spatial use, monitor activity and identify key behaviors such as dustbathing (Sozzi et al., 2022), feather pecking (Subedi et al., 2023), stretching (Li et al., 2021b), eating, resting, and drinking (Fang et al., 2021; Li et al., 2020; Neethirajan, 2022; Ojo et al., 2022). However, the analysis is limited to the camera's field of vision, which can be a limitation in large, complex environments such as farms. In contrast, body-mounted sensors, such as radio frequency identification (**RFID**), ultra-wideband (**UWB**), and accelerometers, are not constrained by the environment. However, the technology is not yet as cost-effective or as easily accessible as cameras. RFID and UWB can provide the location of each bird outfitted with a sensor in laboratory settings, barns and aviaries (Baxter and O'Connell, 2023; Doornweerd et al., 2023; Li et al., 2020; van der Sluis et al., 2020). With accelerometers, high-resolution time series of a wide variety of poultry behaviors can be obtained simultaneously at an individual level, including activity levels (Cassey-Trott, 2018; Dawson et al., 2021; Derakhshani et al., 2022; Pearce et al., 2024; Shahbazi et al., 2023), jumping/landing (Banerjee et al., 2014), dustbathing (Fonseca et al., 2024), male reproductive behaviors (Rossi et al., 2024), as well as other behaviors related to self-maintenance (e.g. grooming), feeding (e.g. food seeking, food and water intake), interactions with conspecifics (e.g. self-defense), and interactions with sensors (e.g. pecking at sensors) (Fujinami et al., 2023; Li and Chai, 2023; Li et al., 2021a; Mei et al., 2023; Pearce et al., 2024; Yang et al., 2021). Thus, an integrated technological approach could provide a comprehensive perspective on animal behavior, welfare and production. The key advantage of integrating various sensors with AI is the potential to offer a real-time and continuous overview of the individual birds' status (Ben Sassi et al., 2016; Rowe et al., 2019) and location (Doornweerd et al., 2024). This would enable rapid interventions that benefit the flocks (Ben Sassi et al., 2016; Rowe et al., 2019) and help to improve poultry health and welfare (Ojo et al., 2022).

Recently, real-time object detection systems such as YOLO ("You Only Look Once") with state-of-the-art performance in terms of accuracy, speed, and network size (Badgujar et al., 2024) have become easily accessible. YOLO can be trained to identify elements in an image, such as chickens, and has been used in poultry houses and coops (Marin et al., 2024; Neethirajan, 2022) and research settings (Sozzi et al., 2022; Subedi et al., 2023; Yang et al., 2021). Moreover, YOLO can be used with a tracking-by-detection algorithm to maintain the identity of a specific animal in a group (Doornweerd et al., 2023; Doornweerd et al., 2024; Jaihuni et al., 2023; Neethirajan, 2022) at least over short intervals of time (Doornweerd et al., 2024). Tracking algorithms are especially powerful if there are phenotypic differences between individuals and groups are small (Okinda et al., 2020). However, within poultry flocks where age, size and color are fairly uniform, maintaining the identity of each individual over prolonged periods of time still remains a challenge for computer vision models. In a recent study, Doornweerd et al. (2024) observed 1,952 losses of identification of individuals (ID-losses) in a flock of 39 broilers during an approximately 2 h test. Their results led the authors to conclude that future studies which require maintaining bird identities (e.g. locomotion) must address not only the issue of the algorithm switching animal's ID and the optimization of the tracking algorithm, but also the necessity of incorporating an external animal identification system (e.g., passive radio frequency identification) (Doornweerd et al., 2024).

The need to maintain the identity of individuals within relatively homogenous flocks for research and management purposes is not a novel problem. This inconvenience in the past has been solved by marking animals with numbered and/or colored tags (Derakhshani et al., 2022; Fujinami et al., 2023; Shahbazi et al., 2023) or even by painting the birds' feathers (Dennis et al., 2008; Marin et al., 2014). However, changes in the phenotypic appearance in poultry and other avian species under certain circumstances have also been proven to alter their behaviors, reproductive success, and social and rank-related interactions (Burley, 1988; Campderrich et al., 2017; Dennis et al., 2008; Johnsen et al., 2000; Johnsen et al., 1997; Liste and Estevez, 2023; Marin et al., 2014). For example, in broilers, increased aggressions toward marked animals have been observed when a small proportion of individuals in the group are marked (Dennis et al., 2008). It is also important to note that birds perceive colors differently than humans, as they not only vary in the sensitivity of their visual cones but also in their density and color processing (Tanaka, 2015). Thus, the particular way in which individuals experience their environment (i.e. "Umwelt"), which depends on the capacity of the sensory organs to generate an interpretation of it, must be taken into account (Bueno-Guerra, 2018). Most diurnal birds have relatively uniform tetrachromatic color vision systems based on four types of single cones expressing four functional options: SWS1, SWS2, Rh2 and LWS (Kelber, 2019). The main variation is found in the SWS1-based pigments, which can either peak in the ultraviolet (**UV**) range between 355 and 370 nm or, as in the case of Galliformes, in the violet (**V**) range between 402 and 424 nm (Kelber, 2019). Therefore, following the conceptualization of the animalś "Umwelt", it is highly relevant to determine how the animal under study may perceive the elements in its environment through its senses (Bueno-Guerra, 2018), in this case, the color of the tags that will be used for their later identification.

The possibility of tracking the position of each animal within a social group over prolonged periods of time (e.g., days or weeks) using AI is an extremely promising prospect for the field of poultry behavior, welfare, and production. How an animal moves and occupies its environment can provide valuable information regarding social dynamics (François et al., 1999). Inter-individual distance maintained between animals depends to a large extent on conflicting tendencies to approach and withdraw from conspecifics (Jones et al., 1999). For example, during agonistic encounters, an individual may increase the distance separating it from others. However, after distancing, social species will also be highly motivated to reinstate social contact by re-approaching conspecifics (François et al., 1999). Spatial usage by each animal can also be assessed using heatmaps, which indicate more or less frequently used areas and provide information regarding potential territoriality (Baxter et al., 2023). Lastly, from spatial coordinates, the locomotor dynamics of each individual can also be studied (Barberis et al., 2023; Guzman et al., 2017; Kembro et al., 2023), offering not only additional information regarding social structures (Alcala et al., 2019; Guzman et al., 2013) but also indirect information regarding health and welfare. Fearful, sick, or injured animals tend to remain less active than healthy, non-fearful birds (Jones, 1996).

We propose using 7 colored (black, gray, white, orange, red, purple, and green) tags in the form of backpacks that can be detected by AI models like YOLO through top-view videos of the pens. These backpacks were designed to hold small light-weight accelerometers; thus they could be used both for marking individuals and for accelerometer recordings. Previous studies have shown that black backpack tags do not significantly affect Japanese quail behavior after habituation (Rossi et al., 2024). However, as stated previously, a bird's perception of certain colors could affect its behavior. In this study, through independent experiments, we aimed to: i) evaluate the avian visual perception of the colored tags; ii) assess the potential effects of tag colors on social behavior; and iii) test the AI model's ability to distinguish and track different color tags on birds within social groups.

Materials and methods

Japanese quails (*Coturnix japonica)* were used as the animal model. Experiments were conducted according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institute of Health (NIH, publication 85-23, revision 1996), and the experimental protocol was approved by the Institutional Animal Care and Use Committee (CICUAL) of the Institute for Biological and Technological Research (IIByT, UNC-CONICET) Acta n° 28.

Animals and husbandry

After hatching, 200 chicks were housed in 15 breeding pens of 90 cm x 45 cm x 45 cm (length x height x width) with a plastic floor covered with rice husk substrate and wire mesh walls. Chicks were initially housed in mixed sex groups of 50 to 60 individuals. Each breeding pen was equipped with a brooder plaque measuring 20 cm x 30 cm (width x length) with an adjustable height. The brooder consists of a thermostatic metal plate calibrated at 37°C, surrounded by fabric curtains that prevent heat loss. This system allows the chicks to freely move in and out from underneath the brooder, helping them regulate their body temperature. The room temperature was set at 24°C. The quails were subjected to a daily 14:10 h light/dark regime under LED lighting with an intensity of 300 lux, and food and water were supplied ad libitum. Birds were monitored daily. At 28 d of age sex was determined by plumage coloration and males were removed from pens. Stocking density was adjusted to ensure that only 8 females remained in each pen (500 cm^2/bird), exceeding minimum space breeding recommendations (El Sabry et al., 2022), and the brooder plate was removed. At least 1 week before testing, stocking density was further adjusted to either 3 or 5 females according to each experimental procedure (see details below). Testing was performed on birds between 150 and 180 d of age.

Colored accelerometer tags

Previous studies have shown that a backpack-type tag is a suitable method for holding accelerometers in behavioral studies (Banerjee et al., 2014; Fonseca et al., 2024; Rossi et al., 2024; Fujinami et al., 2023; Shahbazi et al., 2023; Simian et al., 2022). Plastic backpacks were 3D printed in seven different colors to identify animals in behavioral studies: black, white, gray, orange, red, purple, and green (Fig. 1).

Experiment 1: avian visual perception of colored tags

Five feathers were manually extracted from the dorsal/mantle area of five randomly selected birds between 150 and 180 d old and placed in sealed plastic Ziploc bags. Feathers from the same bird were mounted one on top of each other. The coloration of each bird's feathers and the backpacks of each different color were measured using an Ocean Optics USB4000 spectrophotometer (Ocean Optics, Inc., Florida, USA), with data acquisition performed via SpectraSuite software (Ocean Insights, Rostock, Germany). Measurements were performed every 0.20 nm using a USB2000 miniature fiber optic spectrophotometer (Ocean Optics, Inc., Florida, USA) with a deuterium-tungsten halogen lamp to provide standardized illumination and a UV-visible reflection/backscatter probe with a wavelength range between 300 and 1100 nm. The probe was inserted into a rectangular prism holder at 45° to avoid specular reflections with its head placed at the bottom of the prism at an approximate distance of 3 mm from the sampled surface. Reflectance was measured relative to a white standard (Ocean Optics, WS-1-SS White Standard) and dark standard (lamps switched off and probe covered); both standards were reset between measurements to account for environmental fluctuations. Backpack samples were taken from the top view, as this was visually observable during experimentation.

Spectra were imported in R, trimmed to the wavelength interval of 300 to 700 nm, and subsequently smoothed (Maia et al., 2019). For each spectrum, colorimetric variables (hue, brightness and saturation) were calculated. To quantify the visual stimulation generated by the colors to an avian viewer, spectra were converted to quantum catches using *Pavo cristatus* cone values, and chromatic (**dS**) and achromatic (**dL**) color contrasts were calculated between feathers and backpacks using the functions *vismodel* and *coldist* of the R package pavo: Perceptual Analysis, Visualization and Organization of Spectral Colour Data (Maia et al., 2013; Maia et al., 2019).

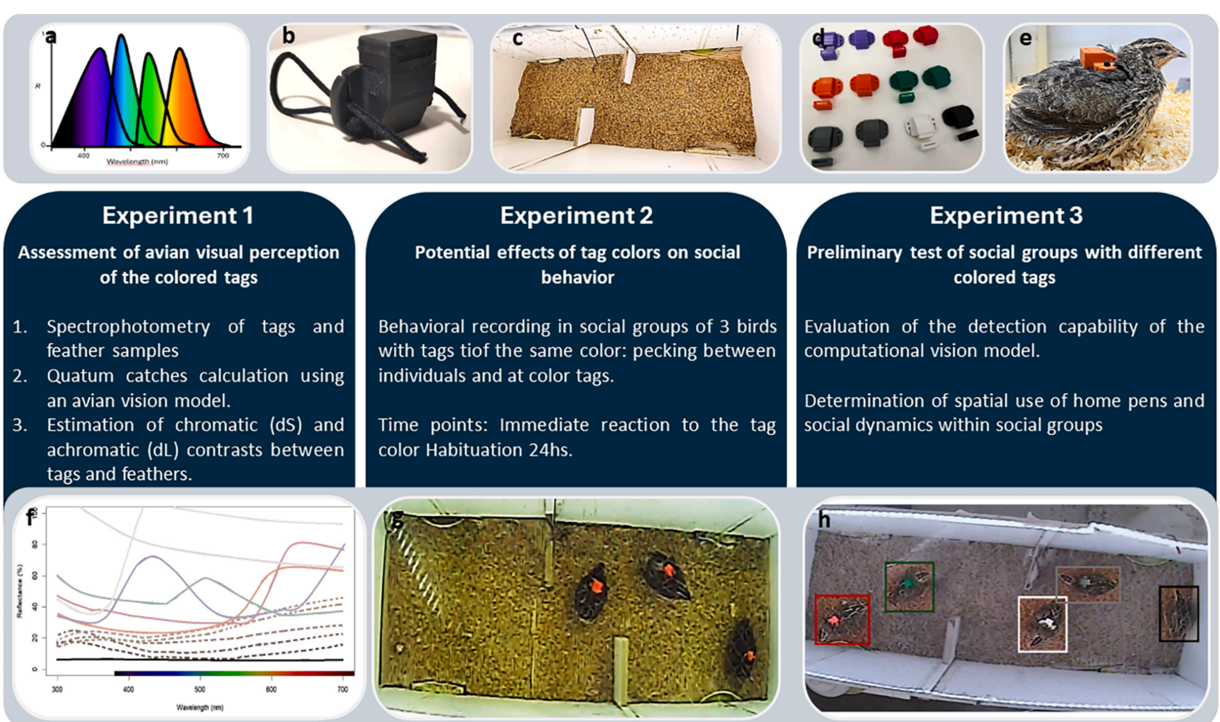

Fig. 1. Experimental protocol. (a-e) Top panels represent photographs of experimental equipment. (a) *Pavo cristatus* visual model adapted from (Hart, 2002; Vorobyev, 2003). (b) Example of black backpack tag. (c) Experimental pen. (d) Color tags. (e) Female Japanese quail with orange tag. (f-h) An illustrative image as well as a brief description of each experiment is provided. (f) Reflectance spectra of backpacks (solid line, with colors representing the color of the tag) and feathers fom different birds (discontinuous lines). A larger version of this panel is provided as Supplementary Fig. 1. (g) Experimental pen with three quails with an orange label. (h) Experimental pen with five quails with different colors tags (white, black, gray, red, and green).

Experiment 2. Social behavior in groups with uniformly colored tags

In this experiment, 35 groups of 3 adult females between 150 and 180 d old were tested. First, during an initial week-long habituation period, each individual was fitted with a black backpack (Fig. 1b) (Fonseca et al., 2024). After this habituation period, 5 groups were moved to the experimental room, and placed in experimental pens of 118 cm x 45 cm x 45 cm (length x height x width), with white melamine walls and floor. The floor was covered with cardboard and rice husk substrate. The experimental pens were provided with 2 tolva-type feeders and 2 bell-type drinkers, positioned on the four sides of the pen, as shown in Fig. 1c. In addition, they had two 13 cm x 15 cm visual barriers in the middle of the box. A suspended camera was placed 1 m above each experimental pen (Fig. 1c). All cameras were connected by closed-circuit to a DVR. Each group was randomly assigned to one of the tag colors (black, white, gray, orange, red, purple and green; Fig. 1d) and their initially fitted black tags were replaced with the newly assigned color tags (Fig. 1e,g). All tests began at approximately 14:00 h. The females remained undisturbed until 12:00 h the next day. This procedure was repeated until all 35 groups were tested, prioritizing that different color groups be represented in each repetition.

From the video recordings, a behavioral analysis of the birds was carried out immediately after the colored tags were placed on the birds. Social interaction time series were obtained by visually observing video recordings and using the ANY-maze™ (Illinois, USA) interface to register pecking behavior. For each bird, a behavioral event was recorded by pressing the corresponding key continuously while the bird was performing the specific behavior and releasing the key once the behavior ended. The number of events of the following behaviors were obtained for each individual within the group:

- Pecks at conspecifićs tag: the bird uses its beak to touch the conspecific's tag (Simian et al., 2024).
- Pecks at own tag: during preening intervals, the bird turns its neck and pecks at its own tag (Simian et al., 2024).
- Pecks performed towards conspecifics: the bird uses its beak to touch a conspecifićs head or body in an area where the tag is not positioned (Caliva et al., 2019).
- Pecks received from conspecifics: the bird receives pecks from conspecifics on its head or body in an area where the tag is not positioned (Caliva et al., 2019).

The observer analyzed each video three times, once for each animal in the group. The animals were previously identified by their location in the pen at the beginning of the analysis. All data analysis and technical validation were performed by a trained and experienced observer (Rossi et al., 2024).

Experiment 3. AI tracking and social dynamics in groups with heterogeneous tag colors

The experimental setup was similar to that of Experiment 2, with a few key differences. Ten groups of 5 adult females were tested. After the habituation period with black tags, each bird had its tag replaced so that all individuals in the same group had different color tags (Fig. 1h). In addition to black tags, white tags were also employed as controls due to their high contrast with the birds' feathers. This resulted in the following combination, where each of the other colors (i.e., gray, orange, purple, red, and green) are represented equally and no colors are repeated within a group:

(1) White – Black – Gray – Orange - Purple.
(2) White - Black - Gray - Orange - Green.
(3) White - Black - Gray - Red - Green.
(4) White - Black - Orange – Purple - Red.
(5) White - Black – Red - Purple – Green.

Each combination was tested twice, resulting in 10 experimental groups. All tests began at approximately 14:00 h, and birds remained undisturbed during the following 4 d period. As explained below, these video recordings were used both to test the capability of the AI tracking algorithm to identify quails according to the tag color and to evaluate the temporal dynamics of spatial use and social dynamics within the groups. For this, video-recordings were converted into images at 1 s intervals for behavioral analysis and variable estimation. As detailed below, these images were analyzed automatically using a computer vision model based on Li et al. (2023) and used in Marin et al. (2024). Specifically, version 9 of the oriented object detection model, YOLO was used (Wang and Liao, 2024), see Section Analysis of videos using AI tracking algorithm for further details. As stated previously, YOLO is a popular object detection model with stable and accurate detection performance that has been previously used for detecting poultry images (Doornweerd et al., 2023; Yang et al., 2022; Doornweerd et al., 2024; Guo et al., 2023; Li et al., 2023). The computer vision model required prior training and validation, for which a separate set of images was obtained from videos as explained in the next section.

Training and validation of AI tracking algorithm for detection of quail with colored tags in images

Twenty-two 30-min videos were randomly selected, assuring that all ten groups were represented at least twice and that the recording had been performed during the daytime. From these videos 791 images were obtained for the training and validation set. RoboFlow (Roboflow, Inc., Lowa, USA), a platform for creating, training and deploying computer vision models, was used. Labeling for object detection (i.e., quail with each tag color) was performed in Roboflow using a horizontal rectangular bounding box. The dataset was split into three groups: 60% for training, 27% for validation, and 13% for testing. The model was trained and validated on Roboflow using the web interface. Preprocessing steps only included auto-orient and resizing (stretched to 800 $\times$ 600). No augmentations were applied. The code is available upon request to authors.

Performance evaluation

To determine the performance of oriented object detection, precision, recall, F1 score, and the average precision metric (**mAP**) were estimated across all classes in model as follows:

$$Precision\ (\%) = (100 \times True\ positive)/(True\ positive + False\ positive)$$

$$Recall\ (\%) = (100 \times True\ positive)/(True\ positive + False\ negatives)$$

$$F1\ score = 2 \times ((Precision \times Recall)\ /\ (Precision + Recall))$$

$$mAP = \frac{1}{N}\sum_{i=1}^{N} AP_i$$

where "True positives" is the number of cases in which both algorithms and labels show bird presence; "False positives" is the number of cases in which algorithms wrongly predicted bird presence; and "False negatives" is the number of cases in which algorithms wrongly predicted bird absence. The mAP averages over the average precision of each class AP_i, where the subindex i runs over each class corresponds to black, white, gray, orange, red, violet, and green color tags, thus N=7. For estimations, an intersection over union (**IoU**), indicating the overlap of the predicted bounding box coordinates to the labeled box (ground truth) was 0.7, and the confidence level 40. The developed computer vision model was saved as a .pt file for use in further behavior analysis.

Analysis of videos using AI tracking algorithm

A 1 h time frame between 8:00 and 9:00 h on the last day of testing was analyzed. The computer vision model was deployed in Python's Integrated Development and Learning Environment (Python 3.11.6, Delaware, USA) in a local machine with the processor Intel® Core™ i7-8700 CPU @ 3.2 GHz, RAM 16 GB. Images extracted from video recordings at 1 s intervals underwent processing through the trained and validated computer vision model. The model generated detailed information in the form of rectangular bounding boxes encompassing each bird with a colored tag, including the specific parameters X_{min}, Y_{min}, box width, and box length. Furthermore, the coordinates for the center of each bounding box were computed on both the x- and y-axes, representing the centroid coordinates for every bird in the image (Marin et al., 2024). The centroid coordinates obtained for each animal in the frame was used for tracking. From these coordinates the following variables were estimated:

- Correct identification (%): the percentage of 1 s time intervals in which the model detected the tag color only once. If the tag was detected twice within the same interval, even if bounding boxes overlapped, it was not considered a correct identification.
- Inter-individual distance (cm): the Euclidian distance between the centroid coordinates (x,y) of each pair of birds estimated in an image. For example, if the coordinates for bird 1 are (x_1,y_1) and for bird 2 are (x_2,y_2), the distance between them in pixels can be calculated using the following formula (Guzman et al., 2013):

$$\sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$$

- This measurement is then transformed to cm, using the length of the experimental pen as a scale to estimate equivalence between pixel and cm (Guzman et al., 2013).
- Average inter-individual distance (cm): the average distance between two birds during the 1 h test period.
- Social circle (cm): the average value of social distance between all birds in the 1 h test period.
- Speed (1 cm/s): the change in position of the animal (centroid coordinates, x,y) during a 1 s interval (i.e., velocity). For example, considering the centroid coordinates for bird at time t as x_t,y_t and after 1s as x_{t+1},y_{t+1}, the following formula calculates the distance between them in pixels (Barberis et al., 2023; Doornweerd et al., 2024; Okinda et al., 2020):

$$\frac{\sqrt{(x_t - x_{t+1})^2 \; + \; (y_t - y_{t+1})^2}}{1}$$

This measurement is then transformed to cm, using the length of the experimental pen as a scale to estimate equivalence between pixel and cm (Barberis et al., 2023).

- Average speed (cm/s): the average speed of the birds during the 1 h test period.
- Ambulation (%): Percent of time during the 1 h test period in which the animal showed speeds above 1 cm (Barberis et al., 2023; Caliva et al., 2019).

Heatmaps of the localization of each animal within pens were constructed using two-dimensional kernel density estimation (Fernandez et al., 2021; Kembro et al., 2019). This process specifically utilized the *ksdensity* function in MATLAB version R2023a, 9.14.0.2337262 (MathWorks, Inc., Massachusetts, USA), and the coordinates of the animals obtained during the 1 h test period.

Statistical analysis

All statistical analyses were performed with R software using the library lme4, and glmmTMB and glmer functions. Generalized Linear Mixed Models (GLMM) were used to determine behavioral differences in Japanese quail associated with the use of color marking. In experiment 2, a ZERO-Inflated Model component was incorporated into the model considering the structure of the data. In this case, the model was adjusted to a Negative Binomial distribution for all variables, taking as a decision criterion a lower AIC compared to other distributions. In experiment 3, the data for speed was fitted to a Gamma and the percentage of ambulation were fitted to a Normal distribution. For all analyses, experimental batches and experimental pens were used as random factors and tag color as a fixed factor. A *P*-value of less than 0.05 was considered to represent significant differences.

Results

The spectrum of each feather and tag is provided in Fig. 1f and in Supplementary Fig. 1. Feathers exhibited a brightness between 10.9 to 29.5%. Contrarily, backpacks showed variability in their level of brightness with black showing the lowest levels and white and gray showing the highest levels (Table 1).

The quail's perceived difference between feathers and each tag color in terms of dS and dL contrasts are shown in Fig. 2. The greatest dS contrast to feathers was observed by purple and green (Fig. 2a). White, black, gray, orange and red tags fell below the threshold for optimal chromatic perception of 5 **JND** (Just Noticeable Differences; Fig. 2a, red line). Regarding dL contrasts, variability was observed between the feathers from each bird and tags. In general, tags showed greater luminance receptor stimulation than feathers (Fig. 2b), surpassing the 5 JND threshold. Thus, birds should easily perceive the achromatic contrast between feathers and tags. However, in 2 out of 5 birds, orange, purple, red and green tags showed dL values below the threshold for achromatic perception (Fig. 2b, red line). Also, for the black tag 1 out of the 5 birds showed dL values below the threshold.

In the second experiment, the pecking behavior of individuals within groups of homogeneously colored tags was assessed, and the results are shown in Fig. 3. Significant effects of tag color ($P<0.05$) were observed for Pecks at own tag (Fig. 3a), Pecks performed towards conspecifics (Fig. 3c) and Pecks received from conspecifics (Fig. 3d) but not for Pecks at conspecifics tag (Fig. 3b). Specifically, quail with white ($P=0.0007$), gray ($P=0.039$), violet ($P=0.024$), and green ($P=0.022$) backpacks pecked more at their own tags compared to those with the control black tags (Fig. 3a). However, birds in groups with orange tags performed and received fewer pecks ($P<0.01$) directed at the head or body compared to those in groups with other colored tags (Fig. 3c and d, respectively).

In the third experiment, the performance of YOLO for tag color identification in images was assessed. The estimated Precision, Recall and mAP was 95.9%, 95.9% and 97.3%, respectively. Fig. 4a shows the Confusion Matrix estimated during validation, which quantifies how many times the algorithm assigns the color correctly. Note that for all tag colors, the probability of true positives (diagonal) exceeds 0.9 (i.e., 90%). The highest level of false predictions occurs between black and

Table 1
Ranking of Backpack tag colors according to brightness from lowest to highest values.

Rank	Backpack color	Brightness (%)
1	Black	6.2
2	Orange	38.7
3	Green	44.7
4	Red	45.2
5	Violet	46.0
6	Gray	79.0
7	White	84.0

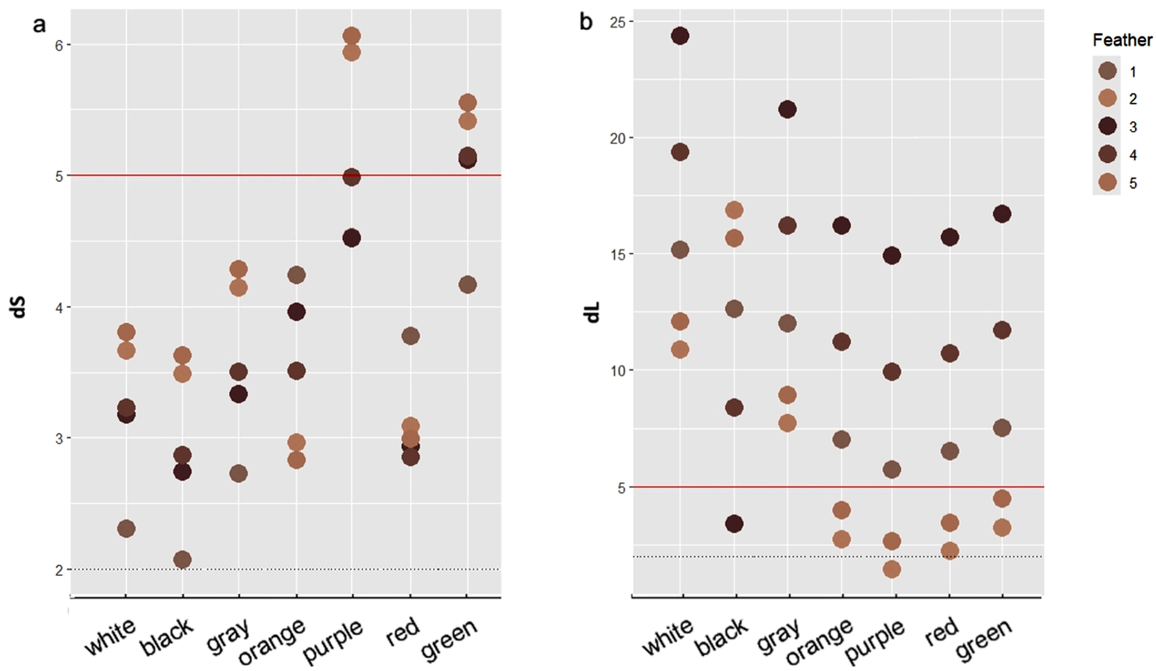

Fig. 2. Chromatic (dS) and achromatic (dL) color perception contrasts between colored backpack tags and feathers. Feathers were obtained from 5 different female Japanese quail as indicated with the circle color. The red line in both panels indicates the threshold of 5 JND (Just Noticeable Differences) above which the bird is considered to perceive the contrast between the feather and tag.

gray tags, with 6% of false positives. Misclassifications with the background are most frequent for black tags, followed by purple and gray tags.

To further understand how the combination of tag colors could affect individual identification within social groups using YOLO over time, we ran the model on 1 h-long behavioral videos, sampling every second. Fig. 4b shows the percentage of overall correct identifications done by YOLO for each tag color across the 10 social groups studied. All colors, except black and gray, showed over 90% correct identification in all groups.

Fig. 5 provides examples of behavioral estimations calculated using the spatial coordinates of each bird with a colored tag. The distance between each bird in each frame was measured (Fig. 5a) and subsequently plotted as a function of time (colored lines in Fig. 5b). From this inter-individual distance, the average value was estimated representing the social circle (black dotted line in Fig. 5b). From these time series it is evident that in groups, some birds stay predominately far away from each other, while others stay in close proximity to each other (Fig. 5b, d). This is quantitively displayed in Fig. 5d, where the average distance between each bird in the example social group is shown in a heat map with red indicating birds stayed further apart than the estimated social circle and blue indicating they stayed closer. The ambulation of each bird is shown in Fig. 5c. Note that, in this social group example, most ambulation occurred in bouts with most animals moving around at the same time. Lastly, the heatmaps of spatial localization of each bird from the example group are presented in Fig. 5 e-i. Some of the animals stay predominantly on a certain side of the box since water and food were available at each side. For example, the bird with the black tag predominantly stays on the left (Fig. 5f) and the one with the red tag on the right side of the box (Fig. 5i), which is consistent with an average inter-individual distance of 81 cm (Fig. 5d).

Lastly, in these multi-colored tag social groups, speed (Fig. 6a) and percentage of time spent ambulating (Fig. 6b) by each individual were assessed according to tag color. Although inter-individual differences are apparent, they are not associated with tag color ($P>0.10$). Average inter-individual distances for all color combinations are presented in Supplementary Fig. 2.

Discussion

Artificial marking of animals for identification is frequently employed by researchers in the behavioral, biomedical, agricultural, and environmental sciences (Dennis et al., 2008). In the context of precision farming, studies have relied on marking backpacks with different colors for identification of individuals within flocks (Derakhshani et al., 2022; Fujinami et al., 2023; Shahbazi et al., 2023). However, to the best of our knowledge, this is the first study to evaluate the impact of backpack tag color on poultry behavior. We show that care should be placed on the color selection of tags, not only because certain colors such as white, can lead to more birds pecking at their own backpacks, but also because certain color combinations could be prone to identity switches by the AI algorithm YOLO (i.e. black/gray).

Birds' color perception abilities significantly influence behavioral experiments in ornithology, as their responses to color cues are context-dependent and shaped by ecological/evolutionary factors. Galliform (eg., Japanese quail, chicken and peafowl) photoreceptors are characterized by a violet spectrum (λ_{max} 415 to 426 nm) visual pigment in the single cone containing a transparent T-type oil droplet. Moreover, excluding double cones, the potentially tetrachromatic color vision system of the peafowl (Fig. 1a), has three spectral loci of maximal wavelength discrimination, where the cone spectral sensitivities overlap at approximately 462, 517 and 576 nm (Hart, 2002). This enhanced color vision requires careful consideration in experimental setups, as the color stimuli must align with the birds' perceptual capabilities to yield valid results (Garcia et al., 2021). Moreover, studies have shown that color preferences can vary widely among species, impacting their responses to visual cues (Kelber, 2018). For instance, certain colors may attract specific species more effectively, which can skew data if not properly accounted for (Aviles et al., 2010). Therefore, researchers must tailor their experimental designs to incorporate the specific color perception profiles of the target species, ensuring that findings accurately reflect their natural behaviors and preferences (Olsson, 2016).

Using an avian vision model, we show that from a chromatic standpoint, only green and purple tags present dS values above the 5 JND threshold. This indicates that Japanese quail are able to easily

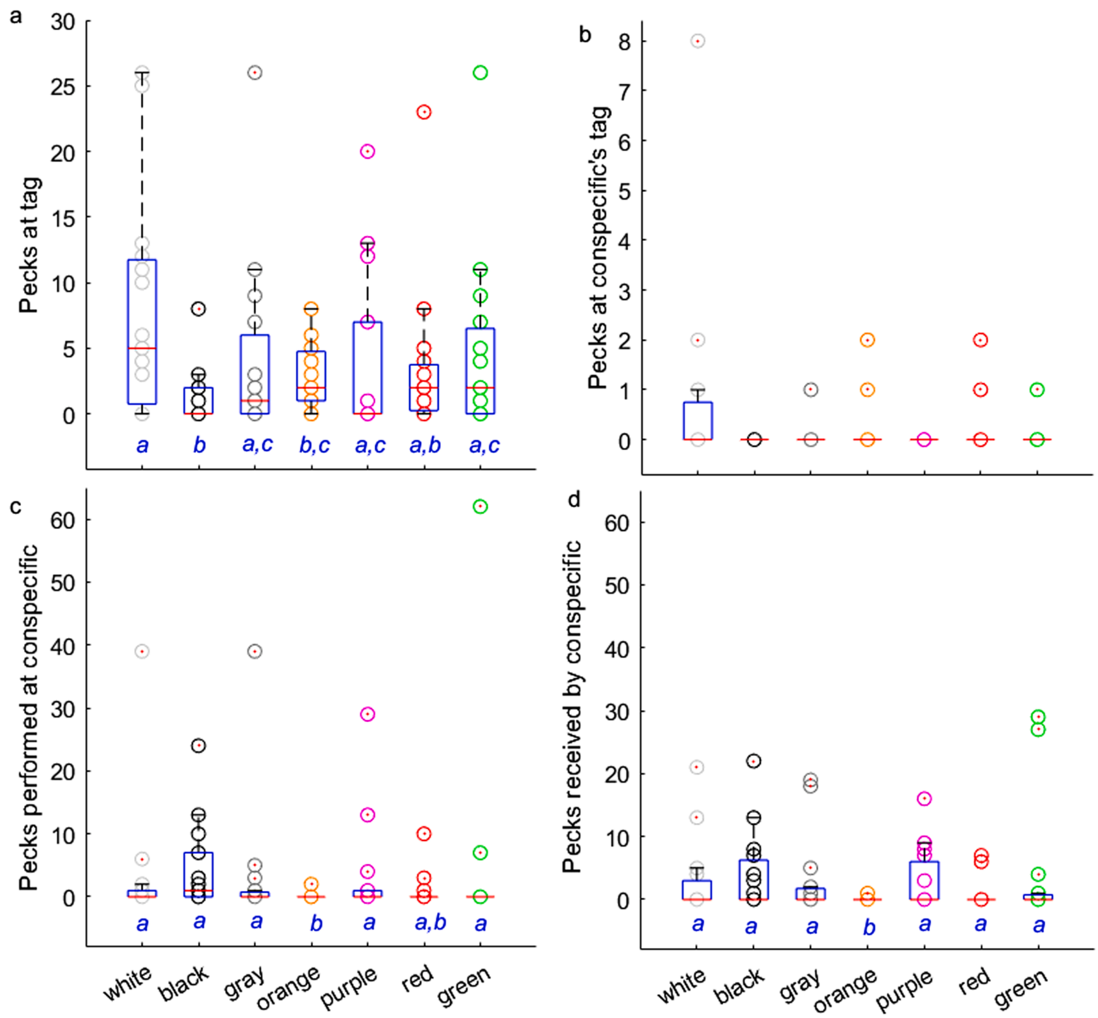

Fig. 3. Pecking behavior performed within homogeneous colored tag groups. Number of (a) pecks performed towards their own accelerometer. (b) pecks performed towards the accelerometer of conspecifics, (c) pecks performed towards the head or body of conspecifics and (d) pecks received by conspecifics directed to the head or body. Each individual is represented with a circle, for boxplots, boxes represent 25th and 75th percentiles of the sample data, the middle red line the 50th, and the whiskers the 1.5 times the interquartile range. [a-c] Post hoc analysis results are shown using blue lower-case letters. Groups that do not share the same letter differ significantly ($P<0.05$).

perceive the contrast between the feather and these tag colors (Fig. 2a). However, from an achromatic (luminance) standpoint higher variability was observed between the feathers of different birds and tag colors. In general, with some exceptions, the tags presented dL values above the JND threshold, thus presenting a perceivable achromatic contrast to feathers. Interestingly, in certain females, the orange, red, or black tags showed neither chromatic nor achromatic contrast with their feathers. This lack of contrast suggests that these tags could remain inconspicuous in quail with specific plumage characteristics, offering the advantage of going undetected. In this context, it should be noted that, besides the wild-type plumage coloration studied herein, several other quail plumage colors occur naturally by mutation of single genes, e.g. extended brown, yellow, silver, lavender, roux, imperfect albinism and rusty (Minvielle et al., 2009). These feathers can differ significantly in melanin and phaeomelanin content (Minvielle et al., 2009). Plumage color mutants, such as “orange” present light reddish-brown and light black predominantly, instead of the heavy brown and heavy black of the wild type (Ito and Tsudzuki, 1994). Thus, although not specifically evaluated herein, some of the colors tested, such as black, white and orange, could show less contrast to feathers in some of these lines, or with feathers from other parts of the body not analyzed in this study.

Although most tags presented either a chromatic or luminance (achromatic) contrast with feathers perceptible to birds, the effect on behavior was color dependent. Even when the chromatic contrast, dS, fell below the JND threshold, birds with higher dL and brighter backpacks tended to peck more at their backpacks, as in the case of white and gray tags. This suggests that the brightness of the material could be important in modulating pecking behavior. Indeed, white backpacks were the brightest 84%, Table 1), with a reflectance above 90% from 450 to 700 nm (Supplementary Fig. 1), presented the greatest achromatic contrast (dL) and presented the highest level of self-pecking. It is noticeable that white is a naturally occurring feather color in quails. Females have predominately white feathers on their breast; and white flight feathers are observed in the albino quail, in dotted white mutants (Tsudzuki et al., 1992). Males during winter season as well as photocastrated males are also provided of white breast feathers (Roberts et al., 2009). In groups of wild-type quails, social interactions towards the albino’s quail have been shown to be different than towards their wild-type counterparts. For example, Blohowiak and Siegel (1983) showed that wild-type females prefer mating with wild-type or redhead color males more than with albino males (Blohowiak and Siegel, 1983). In the same study, regarding dominate-subordinate relationships among males within a pen, they observed that redhead and wildtype males dominated their albino flockmates (Blohowiak and Siegel, 1983). Nevertheless, in our study, although birds with white and gray tags were found to peck more at their own tag within their groups, no increases in

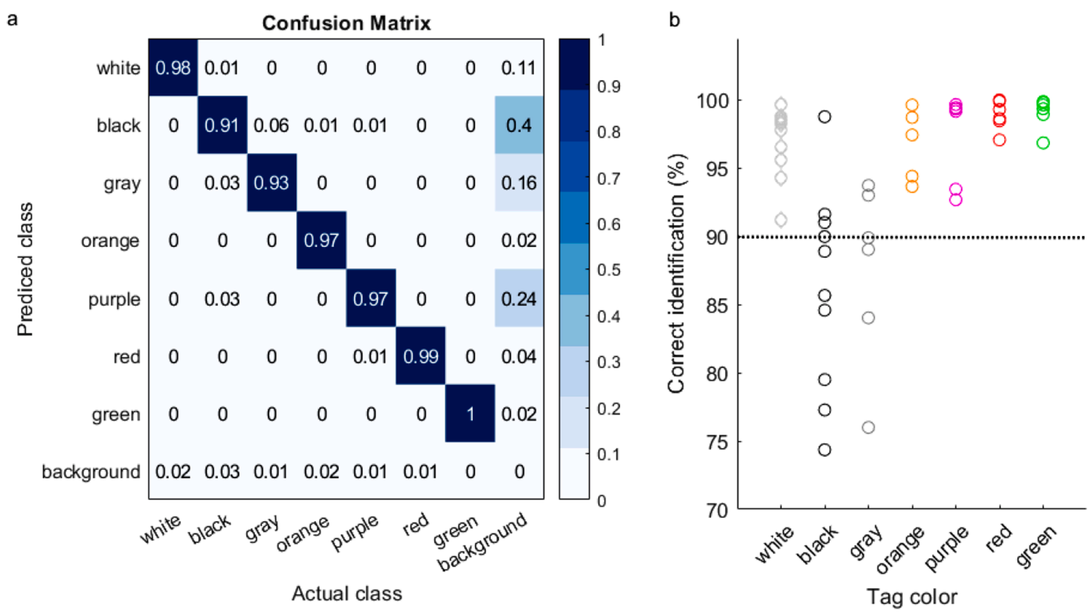

Fig. 4. Detection capabilities of the computer vision model in correctly classifying individuals based on tag color in mult-colored tag social groups. (a) Confusion Matrix was estimated using RoboFlow. (b) Overall correct identification was estimated after running the model on 1 h long behavioral videos, with a 1 s sampling rate. The percentage of overall correct identifications was calculated based on the total time points a bird with a colored tag appeared in the corresponding frame. If the tag was found twice that time point was not counted as a correct identification. Boxplots are presented for each tag color, with each colored circle representing the overall average for each color tag across all 10 groups studied. All colors, except black and gray, showed over 90% correct identification (dotted black line) in all groups.

the number of pecks toward conspecifics were found.

Groups presenting tags with high chromatic contrast with feathers, such as purple and green (Fig. 2a), also presented higher levels of pecking at their own tag compared to groups using the black "control" tags. In contrast, the social groups with animals that orange and red tags, where tags present low chromatic contrast with feathers (Fig. 2a) and low brightness (Table 1) did not present significant differences with black "control" tags. Moreover, those with orange tags even showed significantly lower values of pecking towards and from conspecifics. This phenomenon can also associate with the previously discussed lack of chromatic and achromatic contrast between feathers and the orange, red and black tags observed in some females. Within mixed-colored groups, no effects on speed and ambulation were observed regarding tag color. It is also important to note that none of the groups evaluated presented animals with marked signs of lesions that could be associated with inappropriate (non-adaptive) levels of aggression during the habituation and testing periods. Thus, observed pecks towards tags and conspecifics could mostly be associated with exploratory and/or social interactive/affiliative behaviors. Further studies using less reflective materials for the backpacks, standardized images of birds with backpacks, as well as comparative studies using mutant color quail would be worthy to further explore the bird's perception of the tags in situ.

With the use of color tags, an elevated level of precision and accuracy of the AI models for multi-animal tracking was observed. When they occurred, most misclassifications were found between black and gray tags. In this case, the reduced accuracy could be attributed to the algorithm's heightened likelihood of confusing gray backpacks with black ones, and vice versa, compared to other colors (i.e., white, orange, green, purple, and red). This misclassification likely arises from the similarity between black and gray, making accurate identification more challenging. Thus, combining these two colors should be avoided in future studies. Since the identity of the individual can be recovered in subsequent time points after a misclassification, a correction code can be applied to rectify these errors. Furthermore, the AI model could also be improved by incorporating Multi-Object Tracking "MOT", for example, using the popular tracking-by-detection algorithm Simple Online Realtime Trackin, "SORT" previously used in broilers (Doornweerd et al., 2024).

Multiple animal tracking plays a vital role in predicting animal movement and behavior analysis (Liu et al., 2024). It finds extensive applications in various fields, including agriculture, animal husbandry, and ecology (Liu et al., 2024). However, in complex environments and real farm environments, the task of monitoring poultry becomes complicated. Birds in flocks are sometimes occluded by other birds and the variation of ambient light conditions and shadows significantly affect sensor stability (Okinda et al., 2020). In flocks with unmarked birds, computer vision models ID-switches are a common occurrence (Doornweerd et al., 2024). Thus, colored marks ensure that even when tracking is lost or identifies switches for any reason, the true identity of the bird can be regained minimizing the long-term impact of algorithmic errors.

The coordinates obtained from the multiple animal tracking can be used to calculate the time series of a wide variety of behavioral variables as shown herein: inter-individual distance, spatial use, speed and ambulation. All of these variables can present complex temporal patterns that can change over time. Inter-individual distances are dynamic, and differ according to the behavior displayed (Keeling, 1994) and the stocking density (Keeling and Duncan, 1989; Rodriguez-Aurrekoetxea and Estevez, 2014). Locomotion (i.e., speed and ambulation) presents not only circadian and ultradian rhythms, but also long-range correlations at shorter time scales (Barberis et al., 2023; Guzman et al., 2017; Kembro et al., 2013; Kembro et al., 2023). Quail locomotor dynamics is modulated by factors such as social hierarchy (Alcala et al., 2019), stress (Kembro et al., 2008) and food availability (Kembro et al., 2009). Variables that characterize dynamics, such as rhythms and long-range correlations, are often more sensible in detecting behavioral changes than traditional measures such as the percent of time spent performing a specific behavior (Alcala et al., 2019; Kembro et al., 2009; Kembro et al., 2024; Rutherford et al., 2004). Thus, the ability to correctly keep track of the identities of individuals within social groups over long periods of time opens up a wide range of possibilities for developing new assays for the study of locomotor and social behavior. Since this tracking can be

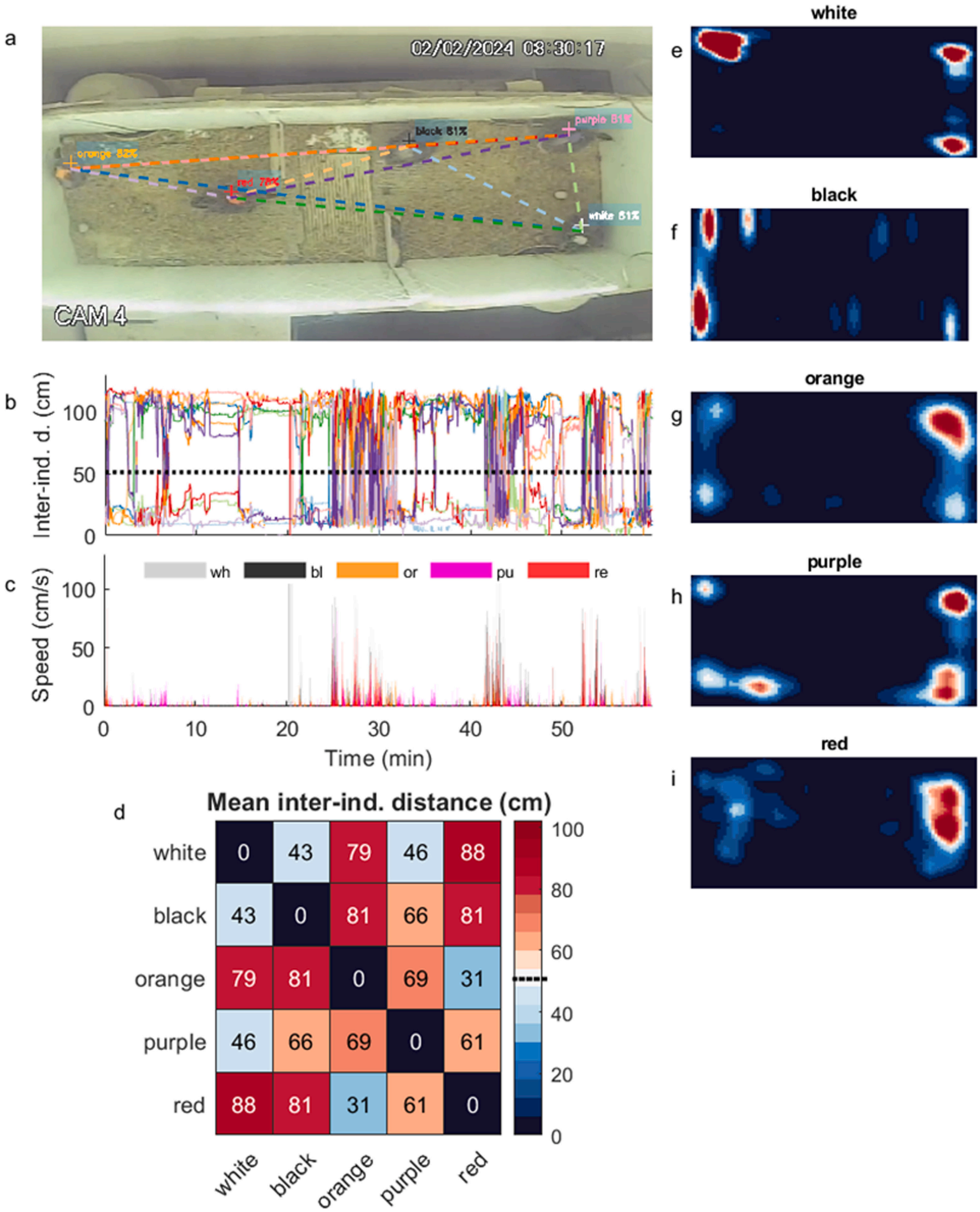

Fig. 5. Examples of behavioral estimations using spatial coordinates of birds with colored tags. (a) Image representing the social distance between each bird in a social group using colored lines at a specific time point. (b) Using the same color scheme as in panel a, the social distance between each individual is shown as a function of time. Thick, dotted black line shows the estimated social circle. (c) The distance ambulated by each individual during the 1 s intervals is shown as a function of time. (d) Heat map of the average social distance between each bird during the 1 h test. Reds indicate birds staying farther apart than the estimated social circle and in blue those closer than the social circle. (e–i) Heatmaps of spatial use of each bird.

combined with accelerometry, the study of the temporal dynamics of behavior can be further extended to include for example reproductive (Rossi et al., 2024) and dustbathing behaviors (Fonseca et al., 2023).

It is important to note that while this study was conducted in a controlled laboratory setting with only seven color combinations, the approach could be applicable to larger, more complex experimental designs. In theory, if individual identification of a large number of birds is required, backpacks with varied color patterns or symbols could be 3D printed. For instance, in a farm setting, a subset of animals could be marked with multi-colored backpacks and identified by strategically placed cameras. Moreover, scaling this method for commercial farm use would require further validation. This includes testing the AI's ability to recognize multi-colored backpacks, assessing the long-term effects of backpack use on animal welfare, and examining the social dynamics within groups where only a subset of animals is marked.

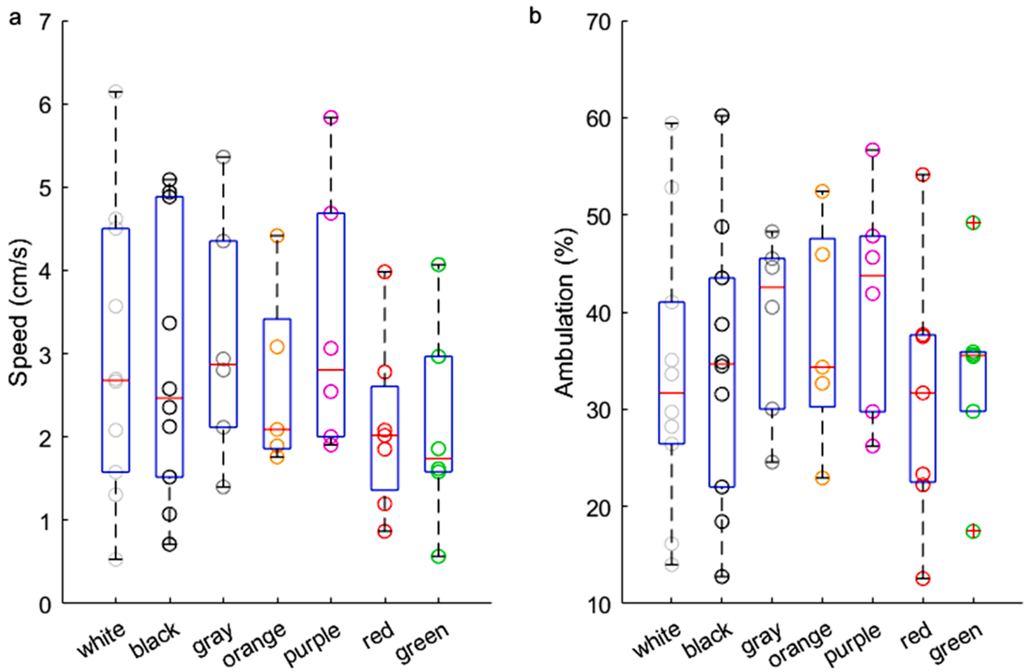

Fig. 6. Dynamics within multi-colored tag social groups. (a) Mean speed and (c) percent of time spent ambulating by each bird within the ten social groups. No significant differences were found between color tags.

Conclusion

Automatically tracking individual birds within social groups using computer vision, combined with other remote sensing technologies like accelerometers, opens new frontiers in poultry behavioral research. This approach allows for long-term, high-temporal resolution studies of social dynamics, reproduction, and the effects of environmental enrichment on locomotor dynamics and spatial use. By assessing both individual and collective behavioral dynamics, welfare studies can account for inter-individual differences in environmental perception and health. As changes in bird behavior often signal welfare issues, early detection is crucial for timely intervention and prevention of management problems.

Declaration of AI and AI-assisted technologies in the writing process: During the preparation of this work the author(s) used chatGPT in order to revise grammar and clarity of key phrases.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2024.104464.

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